

THE EFFECTS OF LOW FREQUENCY NOISE ON PERIODONTAL LIGAMENT— A HISTOMORPHOLOGIC STUDY ON MOUSE

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ABSTRACT

The study was conducted to investigate the effect of low frequency noise on histomorphology of periodontal ligament of mouse. The study was randomized control trial of three month duration.

Thirty adult BALB/c mice, half male and half female, were randomly divided into three groups. Control group A was kept in normal environment of animal house. Experimental group B was kept in silent condition while experimental group C was exposed to low frequency noise of 200 Hz continuously. At the end of three months, mice were euthanized, mandible removed and sections were taken for light microscopic study

Overall there was significant difference when comparing groups A (normal environment), B (silent group), and C (low frequency noise exposed) regarding arrangement of Periodontal ligament fibers, thickness of the ligament, thickness of wall of blood vessels and destruction of alveolar bone. In most of the specimens, distortion of the normal periodontal fiber arrangement was encountered.

It was concluded that exposure to low frequency noise significantly distorts the normal architecture of the periodontal ligament thus causing compromised function of the ligament.

Key Words: *Low frequency noise (LFN), periodontal ligament (PDL).*

INTRODUCTION

Noise pollution is one of the common problems of the urban community of most of the developing and developed countries. The problem has increased by industrialization and large infra-structure networks. The sound and noise are same as far as their mechanical properties like source, propagation and perception is concerned. The difference is that the noise is the undesirable sound causing discomfort to the listener. It travels in the form of pressure waves through a medium.¹

The frequency is defined as the number of oscillations per second, denoted as Hertz (Hz). The audible range of frequency is between 20 to 20000 Hz. Infra sound is the frequency below 20 Hz and ultrasound

is above 20000 Hz.¹ The term low frequency noise is attributed to frequency of ≤ 500 Hz.² Low frequency noise is an essential part of frequencies generated in daily life by automobiles, heavy machinery, fans, generators and aircrafts etc.

Periodontal ligament, an important component of periodontium, is connective tissue between cementum and alveolar bone. The thickness ranges from 0.15 to 0.38 mm in human. It is thinnest around middle third of the root of tooth.³ The main functions of the periodontal ligament include supporting the tooth and acting as sensory receptor. Periodontal ligament is a well vascularized and innervated structure. The structural components include cells (fibroblasts, macrophages and cementoblasts) and well defined collagen fibers embedded in ground substance. The fibers are arranged in definite and distinct bundles. At each end, these fibers are anchored to cementum or bone. These are known as Sharpey fibers.⁴ These fibers are fully mineralized in cellular cementum and partially mineralized in periphery. In addition to collagen fibers, some elastic fibers are also present in Periodontal ligament.⁵

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The effects of low frequency noise on histomorphology, physiology and biochemical parameters have been studied on different organs and systems of body. Vibroacoustic disease (VAD) is defined as pathology involving whole body in response to chronic exposure to low frequency noise. It is characterized by proliferation of extra cellular matrix.⁶ The mechanism of injury can be direct vibrational effect, stress, vascular involvement or combination of these factors.²

Chronic exposure to low frequency noise effects different body systems like endocrine system⁷, cardiovascular system⁸, immune system⁹, reproductive system¹⁰, vestibular system¹¹, respiratory system¹², central nervous system¹³, digestive system¹⁴ and oral cavity.²

The normal architecture of periodontium can be affected by aging¹⁵, stress¹⁶, smoking¹⁷, drugs¹⁸, alcohol¹⁹ and low frequency noise.²

METHODOLOGY

The study was carried out in the Department of Anatomy, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad. The experiment was carried out with the permission of ethical committee of center for research in experimental and applied medicine (CREAM) of the Army Medical College, Rawalpindi.

Grouping of animals

Thirty adult BALB/c mice, half male and half female, weighing 25-30 grams were used in the experiment and were kept in controlled environment of Animal house of National Institute of Health, Islamabad. They were fed with standard National Institute of Health laboratory diet for three months. Mice were randomly divided into three groups (n = 10 animals, half male and half female, in each group). Control group A (kept in normal environment of animal house), Experimental group B (kept in silent conditions) and Experimental group C (exposed to low frequency noise of 200 Hz continuously for three months). Low frequency noise was produced by analogue frequency generator. The frequency output was confirmed with the help of digital universal frequency counter (Thurlby Thunder, model No TF 830, 1.3 GHz) and oscilloscope (Hitachi VC 6155). Frequency and intensity of sound were recorded at start, middle and end of the experiment.

Dissection, fixation, processing and staining

At the end of 90 days, the animals were euthanized by placing ether soaked cotton in the jar.²⁰ The skin was removed with the help of scalpel. Mandible was dislocated at the temporomandibular joint and dissected out. Specimens were fixed in 10 percent formalin

for twenty four hours. 5µm thick coronal and sagittal slices of animal tissue were made and stained with Hematoxylin & Eosin (H&E) for routine histological study.

HISTOLOGIC STUDY

Motic image plus microscope Model DMB3:223 and Micrometry were used for histological study. The observations on each of the parameters were recorded. Slides were studied at 4X, 10X objectives for general architecture and 40X objective for micrometry. The width of periodontal ligament was calculated by using the Motic Images Plus 2.0 ML software on the computer and confirmed with the help of micrometry.

The qualitative examination was done at 4X, 10X, 40X objectives. The slides were studied for general architecture of the periodontal ligament fibers (regular or distorted) and erosion of alveolar bone with detached pieces of bone among the fibers.

The thickness of periodontal ligament was measured at the level of middle of root of tooth by drawing the line at right angle to the surface of the tooth on both sides and then mean was calculated as the reading for that animal.

Thickness of vessel wall in periodontal ligament was also measured. Three blood vessels were selected randomly in each specimen. Number of ocular divisions were counted from outermost boundary of vessel from one side to other and labeled as 'A'. Another measurement was taken from endothelial margin from one side to other in the same way and labeled as 'B'. The difference between A and B was labeled as thickness of wall of vessel. Wall was measured at three levels and mean was taken as reading for that animal (Fig 3). Readings were taken using 40X magnification.

STATISTICAL ANALYSIS

The data were entered for analysis in the computer software SPSS version 18. Descriptive statistics were used to calculate means and standard error of quantitative data and Chi-square tests were applied to measure the level of significance for analysis. Quantitative data was analyzed with ANOVA and student t-test at a confidence limit of 95 percent and p-value of ≤ 0.05 was considered as significant.

RESULTS

CONTROL GROUP (GROUP A)

The mean thickness of periodontal ligament was $85.80 \pm 11.243 \mu\text{m}$ and the mean thickness of arterioles in periodontal ligament was $4.30 \pm 0.823 \mu\text{m}$. (Table 1)

The histological sections of all the components were stained with H&E and examined under light microscope. The junction of periodontal ligament fibers with the bone in most of specimens was irregular with distorted arrangement of fibers at the ends. Blood vessels were found to be irregularly placed among the fibers of periodontal ligament. Alveolar bundle bone showed erosions at multiple sites with detached bony pieces scattered among the fibers of periodontal ligament. (Fig 4).

GROUP B (EXPERIMENTAL)

The mean thickness of periodontal ligament was $103.80 \pm 7.376 \mu\text{m}$ and that of arterioles in periodontal ligament was $2.70 \pm 0.483 \mu\text{m}$.

The junction of periodontal ligament fibers with the bone in almost all of specimens was regular with well-organized arrangement of fibers at the edges. The fibers of the periodontal ligament were seen regularly running from bone to cementum. Darkly stained cells of the periodontal ligament showed regular arrangement within the fibers. Blood vessels were found regularly arranged closer to the bony side of the periodontal ligament. Alveolar bundle bone showed regular attachment of periodontal ligament fibers with smooth margins. (Fig 5)

GROUP C (EXPERIMENTAL)

The mean thickness of periodontal ligament was $89.70 \pm 9.696 \mu\text{m}$ and the mean thickness of arterioles in periodontal ligament was $2.60 \pm 0.516 \mu\text{m}$. (Table 1)

TABLE 1: COMPARISON OF MEAN VALUES AMONG GROUPS A, B AND C.

Group		Thickness of Periodontal ligament(μm)	Thickness of blood vessel in periodontal ligament (μm).
A	Mean	85.80	4.30
	N	10	10
	Std. Deviation	11.243	0.823
B	Mean	103.80	2.70
	N	10	10
	Std. Deviation	7.376	0.483
C	Mean	89.70	2.60
	N	10	10
	Std. Deviation	9.696	0.516

TABLE 2: GENDER WISE DISTRIBUTION OF VARIABLES AMONG GROUPS A, B AND C.

Groups	Gender		Thickness of Periodontal ligament (μm)	Thickness of blood vessel in periodontal ligament (μm)
Group A	Male	Mean	81	4.36
		N	5	5
		Std. Deviation	24.899	1.029
Group A	Female	Mean	90.6	4.22
		N	5	5
		Std. Deviation	7.57	0.576
Group B	Male	Mean	106	1.87
		N	5	5
		Std. Deviation	7.348	0.447
Group B	Female	Mean	103	2.62
		N	5	5
		Std. Deviation	6.782	0.553
Group C	Male	Mean	85.2	2.72
		N	5	5
		Std. Deviation	8.280	0.227
Group C	Female	Mean	96.1	2.85
		N	5	5
		Std. Deviation	8.765	0.463

TABLE 3: COMPARISON OF P-VALUE AMONG GROUPS A, B AND C.

Parameters	P-Value			
	A&B	A&C	B&C	A&B&C
Thickness of Periodontal ligament(μm)	0.00061	0.33547	.00591	.001
Thickness of blood vessel in Periodontal Ligament(μm)	0.046	0.083	0.398	.000

*p-value <0.05 significant

**p-value <0.001 highly significant

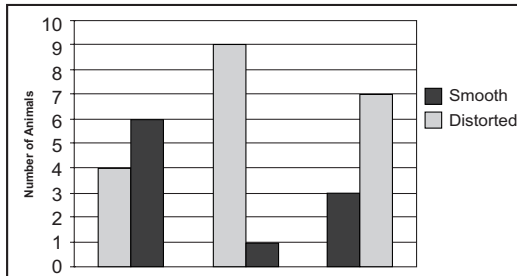


Fig 1: Showing arrangement of periodontal ligament fibers among Groups A, B and C

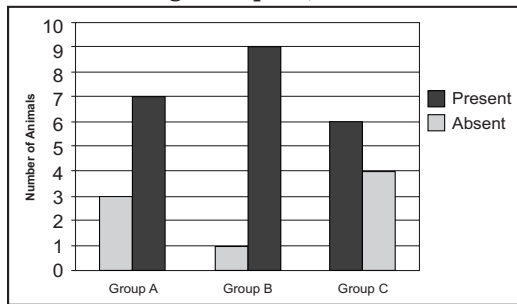


Fig 2: Showing presence of bony fragments among periodontal ligament fibers among Groups A, B and C.

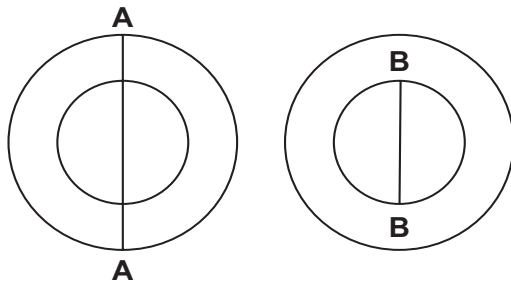


Fig 3: Diagrammatic representation of measurement of blood vessel wall thickness.

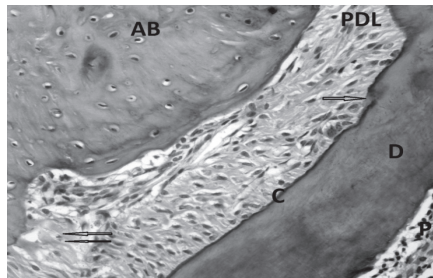


Fig 4: Photomicrograph of sagittal section of animal No 4 of group A showing distorted attachment of periodontal ligament fibers with loss of cells in between (double arrow) and cemental erosion (single arrow). AB: alveolar bone, C: cementum, D: dentin, P: pulp. H&E 40 X.

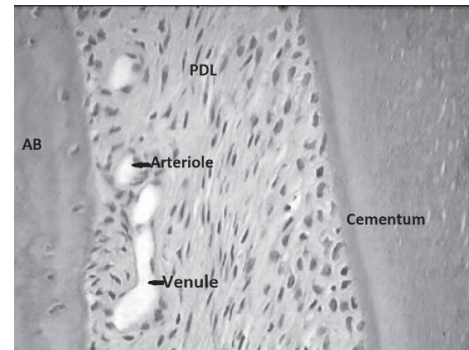


Fig 5: Photomicrograph of sagittal section of group B specimen showing regular arrangement of periodontal ligament fibers with evenly distributed cells in between fibers, smooth margins of line of attachment with alveolar bone (AB) and cementum. Blood vessels arranged along bony side of periodontal ligament. H&E 40 X

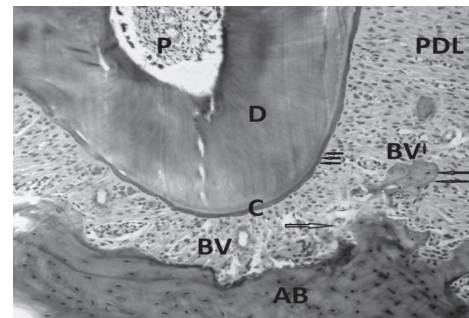


Fig 6: Photomicrograph of sagittal section of specimen from group C showing thick walled arterioles (BV), bone loss (single arrow), detached bone fragments in periodontal ligament (double arrow) and loss of cementum (triple arrow). AB: alveolar bone, C: cementum, D: dentin, P: pulp. H&E 40X

Cementum was seen to be eroded in most of specimens. There was great distortion of attachment of periodontal ligament fibers towards alveolar bone and cementum in all the specimens. Alveolar bone erosion was seen in all the specimens with pieces of bone detached from the alveolar bone seen scattered among the fibers of periodontal ligament (Fig 6). Few osteoclasts were also seen in one specimen. Blood vessels (arterioles and venules) were not regularly placed along the bone margin as in group B.

DISCUSSION

The objective of this study was to see the effects of low frequency noise on the histomorphology of the

periodontal ligament of mice. The results of the experimental groups B & C were compared with those of control group A, as well as with each other.

The mean thickness of periodontal ligament was found to be more in group B than in A & C. (Table 1). The difference was statistically significant when three groups were compared with each other (p value .001). Thickness was more in females than males in group A & C but it was more in males than in female animals in group B (Table 2). Fibers of periodontal ligament were distorted with decreased population of cells. Similar findings were observed by Severson JA *et al* when they compared the architecture of periodontal ligament in young and old age groups.²¹ The wall of the arterioles was found to be thicker in group A & C than group B. It was significantly thicker in group A than B. (p-value .004). The difference in thickness was highly significant (p-value .000) when all groups were compared with each other (Table 3). The thickness was less in group C which contradicts previous studies⁸ done on cardiovascular system.

CONCLUSION

The current study was done to explore new areas in the research world regarding low frequency noise which is present in the normal daily environment. It was concluded that low frequency noise adversely affects the periodontal ligament decreasing its strength and vascularity to perform normal supportive and nutritional functions.

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